

**REMARKS**

Claims 36-42 have been amended and the specification has been amended at pages 13-15 to place the application in better form for examination and to further obviate the 35 U.S.C. §§102 and 112 rejections set forth in the Office Action dated February 7, 2002. It is believed that none of these amendments constitute new matter. Withdrawal of these rejections is requested.

The Examiner has objected to the informal drawings. Applicant is submitting a new Figure 2 under separate cover. Withdrawal of this objection is requested.

Claims 37-42 are rejected under 35 U.S.C. §112, second paragraph as being indefinite. Applicant has amended claims 36-42. Withdrawal of this rejection is respectfully requested.

Claims 36-42 are rejected under 35 U.S.C. §102(a) as being anticipated by Xu et al. Applicant submits that Xu et al. relates to a CT repeat region in the chicken malic enzyme gene promoter. Xu et al. define a minimal region required for the chicken malic enzyme gene promoter activity and perform mutation analysis to determine the importance of certain nucleotides. Promoters were made with 10, 15, and 22 repeats of the CT repeat region. This method is very different than the present invention. Xu et al. do not disclose constructing a synthetic malic promoter which is about 80% homologous over the entire length of the wild type malic promoter. Accordingly, withdrawal of this rejection is respectfully requested.

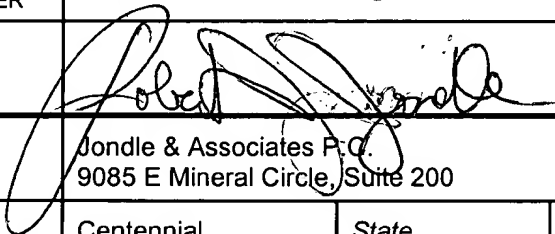
Claims 36-42 are rejected under 35 U.S.C. §102(b) as being anticipated by each of Dickson et al. or Shibui et al. Applicant submits that Dickson et al. Relates to rRNA promoters by mutational analysis. Dickson isolated 290 different mutations of which 112 were examined. They did not construct a synthetic promoter that is about 80% homologous over the entire length of the wild type promoter. Shibui et al. describe the construction of a hybrid promoter "pac" which is comprised of two different promoter regions. The —35 region is from the bacteriophage T5P25 and the —10 region is from the lacUV5 promoter. The "pac" promoter is not 80% homologous over the entire length to either the T5P25 or lacUV5 promoters. Accordingly, withdrawal of this rejection is respectfully requested.

Claims 36-42 are rejected under 35 U.S.C. §102(e) as being anticipated by each of U. S. 5,847,102; US 5,959,094; US 6,162,641; US 6,214,614; US 6,322,962 or US 6,118,049. Applicant submits that US 5,847,102 relates to a hybrid promoter which is a combination of two

different promoters BN115 and CaMV35. This hybrid promoter is not 80% homologous over the entire length to either the BN115 or 35S promoters. Applicant submits US 5,959,094 relates to a promoter of the human p75 TNF-R gene and the first intron. There is no description in the '094 patent relating to making an active homolog with about 80% homology to the promoter. Applicant further submits that US 6,118,049 relates to a hybrid promoter that is a combination of two promoters E8 and E4. The hybrid promoter is not a homolog over the entire length to either E8 or E4. Patent US 6,162,641 relates to a 15 bp sequence in a rat promoter. The short sequence is a response element and not a complete promoter. The '641 patent does not teach how to make an active synthetic homologous promoter. US 6,214,614 relates to DNA repressor elements which are usually 6 or 7 bp in length. These elements are combined with other promoter elements such as transcription initiation sites and enhancers to form a cell cycle regulated promoter. The '614 patent does not teach how to construct a synthetic homologous promoter. Patent US 6,322,962 relates to a sterol-regulated site-1 protease. The '962 patent does not pertain to constructing a synthetic homologous promoter. Accordingly, withdrawal of this rejection is respectfully requested.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned **"Version with markings to show changes made."**

In view of the above amendments and remarks, it is submitted that the claims satisfy the provisions of 35 U.S.C. §§ 102 and 112 and are not obvious over the prior art. Reconsideration of this application and early notice of allowance is requested.

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**Attachments:** Marked-Up Copies of Amendments



**Amended Claims: Version with markings to show changes made**

Please amend claims 36-42 as follows:

36. (AMENDED) A method for preparing a hybrid synthetic promoter that is homologous to a template promoter which comprises the steps of:
- (a) comparing the sequence of a template promoter with known nucleic acid sequences;
  - (b) selecting segments of said known nucleic acid sequences similar to segments of the template promoter sequence;
  - (c) aligning the selected segments in linear order on the basis of the template promoter to derive a first hybrid synthetic homologous promoter;
  - (d) constructing a first hybrid synthetic homologous promoter; and
  - (e) testing the first hybrid synthetic homologous promoter for activity.
37. The method of claim 36, wherein the selected segments have between 60% and 100% sequence identity with segments of the template promoter.
38. The method of claim 36, which further comprises the steps of:
- (f) modifying the sequence of the first hybrid synthetic homologous promoter which does not have maintained or improved activity compared to the promoter to produce a second hybrid synthetic homologous promoter; and
  - (g) testing the hybrid synthetic homologous promoter for activity.
39. The method of claim 38, wherein steps (f) and (g) are repeated until a hybrid synthetic homologous promoter is produced which has maintained or improved activity compared to the template promoter.
40. The method of claim 36, wherein at least 15% of the template promoter sequence has been replaced in the hybrid synthetic homologous promoter.

41. The method of claim 38, wherein at least 15% of the template promoter sequence has been replaced in the ~~hybrid~~ synthetic homologous promoter.
42. The method of claim 39, wherein at least 15% of the template promoter sequence has been replaced in the ~~hybrid~~ synthetic homologous promoter.

**Amended Specification: Version with markings to show changes made**

Please amend the fourth paragraph on page 3 as follows:

Figure 4A and 4B presents a comparison of GUS expression resulting from transiently expressing the gus gene in plasmids p350096 (Fig. 4A) and pMuA0096 (Fig. 4B).

Please amend the second and last paragraphs on page 13 as follows:

The yeast homolog to the CaMV 35S promoter produced a low level of transient expression when compared to the CaMV 35S promoter in transient expression experiments in corn callus (see Example 2). An increase in the level of expression was sought by changing some nucleotides thought to be important for the function of the CaMV 35S promoter. Three bases at positions 277, 278, and 279 of the yeast homolog (Figure 1) were changed from ACA to CGC by site directed mutagenesis using the QuikChange™ site-directed mutagenesis kit according to the manufacturer Stratagene (Figure 1). This new promoter called MuA (Figure 2) in plasmid pMuA0096 resulted in a similar level of transient expression in corn callus when compared to the CaMV 35S promoter (see Example 2, Figure 4A and 4B). The sequence of MuA (Figure 2) has 79.5% homology over 352 base overlap to the CaMV 35S promoter published by Gardner et al., 1981. The comparison of the MuA promoter with the CaMV 35 S promoter is shown in Figure 3.

DNA Delivery: A particle inflow gun (PIG) as described by Finer et al. (1992) and Vain et al. (1993) was used to deliver the DNA. In brief, 50 mg of tungsten particles (M10 from Sylvania Chemicals/Metals, Towanda, PA) were sterilized for 15 minutes in 95% ethanol in a 1.5 ml microfuge tube. Particles were rinsed 3 times in sterile distilled water by repeated vortexing, centrifugation and resuspension in 0.5 ml water. Particle suspensions were made fresh for each experiment. Plasmid DNA was coated onto the particles by mixing 25 ul of tungsten particle suspension (2.5 mg), 5 µl of DNA (5 ug), 25 ul of 2.5 M CaCl<sub>2</sub>, and 10 ul of 100 mM spermidine (free base) . After allowing the particles to settle for a few minutes while on ice, 50 ul of

supernatant was removed. Two ul of the remaining particle suspension was pipetted onto the center of the screen of a syringe filter unit. The syringe filter unit was reassembled and screwed into the ~~Luer-lok~~ LUER-LOK needle adaptor within the chamber. The target tissue in a petri plate was placed about 15 cm below the syringe filter unit. A vacuum of approximately 28 in Hg was applied and the particles were discharged when helium (80 psi) was released following activation of the solenoid by the timer relay.

Please amend the last paragraph on page 14 as follows:

After bombardment, callus was incubated at 25°C in the dark for 16-24 h on the same medium used for bombardment. Then, transient gus expression was evaluated by incubating the tissue in 0.5 mg/ ml X-gluc (Gold Biotechnology, Inc. St. Louis, MO) in 0.1M sodium phosphate buffer pH 7.0 and 0.1% ~~Triton-X-100~~ TRITON-X-100 at 37°C for 4-16 h after which the number and intensity of blue foci were evaluated under a stereo microscope at approximately 10x magnification. Tissue was transformed with either p350096, or pMuA0096. Results are shown in Figure 4A and 4B. It is seen that tissue transformed with p350096 (Fig. 4A) or pMuA0096 (Fig. 4B) had similar levels of transient expression. Tissue transformed with pY0096 was found to have lower levels of transient expression.

Please amend the last paragraph on page 15 as follows:

Immature embryos of Stine elite inbred 963 were aseptically removed from kernels of plants grown in a grow room (15h photoperiod, 28<sup>0</sup> day and 25<sup>0</sup> night). Embryos were harvested 10 to 11 days after pollination when they were between 1 mm and 2 mm in length and then placed in 2 ml of LSinf medium (Table 2) in an Eppendorf tube. The mixture was then stirred with a vortex mixer (~~Vortex Genie 2~~ VORTEX GENIE 2) at full speed for 5 seconds, the LSinf removed, replaced with fresh medium and then stirred again. All medium was then removed from the tube using a Pasteur pipette. Bacteria were collected with a platinum loop (enough to coat the wire of the loop) and thoroughly suspended in 1 ml of Lsinf-AS medium (Table 2) using a Pasteur

pipette. The bacterial suspension was then introduced into the tube containing the embryos and the mixture stirred with a vortex mixer at full speed for 30 seconds. After this the embryos were allowed to stand for five minutes and were then transferred to the surface of LSAS medium (Table 2) solidified with agar, care being taken to remove any accompanying liquid. Embryos were immediately oriented so that the scutellar surface was uppermost.